

ssTEM

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 An abbreviated version of this protocol was published in eLIFE in May 2014

Neuronal connectome of a sensory-motor circuit for visual navigation

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Detailed protocol

This is an updated protocol that works well for *Platynereis* larvae.

- place the planchettes on a Hexadecane-soaked filter paper before making the planchette sandwich for HPF
- freeze sub medium 1% OsO₄ + 0.1% UA in acetone
- Step 1 T-start -90°C, T-end -90°C, time >18h
- Step 2 T-start -90°C, T-end -30°C, slope 5°C/h time 12h
- Step 3 T-start -30°C, T-end -30°C, time 5h
- Step 4 T-start -30°C, T-end 0°C, slope 5°C/h time 6h
- AFS 0°C 4x30 min acetone water free
- AFS 0°C 1x3h Epon 812 - 25% w/o BDMA
- AFS 0°C o/n Epon 812 - 50% w/o BDMA
- AFS room temp 4h Epon 812 - 50% w/o BDMA
- AFS room temp 4h Epon 812 - 75% w/o BDMA
- AFS room temp o/n Epon 812 - 75% w/o BDMA
- AFS room temp 4h Epon 812 - 100% w/o BDMA
- AFS room temp 4h Epon 812 - 100% w/o BDMA
- AFS room temp o/n Epon 812 - 100% w/o BDMA
- AFS room temp 4h Epon 812 - 100% with BDMA
- AFS room temp 4h Epon 812 - 100% with BDMA
- AFS room temp o/n Epon 812 - 100% with BDMA
- polymerization at 66°C minimum 2 days

How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Randel, N. and Jékely, G. (2023). ssTEM. Bio-protocol Preprint. bio-protocol.org/prep2207.
2. Randel, N., Asadulina, A., Bezares-Calderón, L. A., Verasztó, C., Williams, E. A., Conzelmann, M., Shahidi, R. and Jékely, G. (2014). Neuronal connectome of a sensory-motor circuit for visual navigation. eLIFE. DOI: [10.7554/eLife.02730](https://doi.org/10.7554/eLife.02730)

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